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THE NEUROBIOLOGY OF THE ECTONEURAL/HYPONEURAL SYNAPTIC CONNECTION IN AN ECHINODERM

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ABSTRACT

The nervous system of echinoderms consists of two parts, the ectoneural and the hyponeural. The latter is purely motor in function and of mesodermal origin. It is separated from the main ectoneural nervous tissue by a true basement membrane of collagenous connective tissue. Previous anatomical work has suggested that a chemical synapse occurs across the basement membrane with the hyponeural neurons being post synaptic. The brittlestar *Ophiura ophiura* contains very large ectoneural interneurons and hyponeural motor neurons. This report describes for the first time the use of intracellular recording electrodes to dye-fill the cells and monitor action potentials and synaptic potentials. The synaptic morphology of both ectoneural and hyponeural neurons is described from dye-filled cells and shows that the large axons break up into a fine varicose plexus at the terminal regions. Preliminary recordings have been made from ectoneural neurons. Further work is required before function at the cellular level in this nervous system is understood. The report describes in detail, using simultaneous recording from two electrodes, how activity within the ectoneural system drives the hyponeural motor system and thus produces muscular contractions in the intervertebral muscles.

INTRODUCTION

The major structures of the nervous system of eleutherozoan echinoderms consist of the radial nerve cords and the circumoral nerve ring. There are two distinct parts, separated by a basement membrane: the oral ectoneural region and the aboral hyponeural region (Hyman, 1955).

Previous accounts (see Smith, 1966) have described the circumoral ring as a C.N.S. which contains behavior-coordinating centers, but there is no physiological evidence for this and recent anatomical evidence does not support it (Cobb and Stubbs, 1982). A different organization based on anatomical descriptions and recent extracellular neurophysiological work has been proposed (Cobb, 1982). In this scheme it is suggested that whole animal behavior is coordinated by patterns of activity which may be generated peripherally in any one of the radial nerve cords and which activity is propagated virtually unchanged throughout the nervous system (see Stubbs, 1982; Moore and Cobb, 1985). The circumoral nerve ring thus acts as a relay between the arms and not as a centralized 'brain.'

The small size of echinoderm neurons has not previously allowed repeatable extracellular unitary potentials to be recorded and there are no previous reports of intracellular techniques being used. Recently, however, a system of giant fibers has been described in a species of brittlestar (*Ophiura ophiura*) with axons up to 10 μ in diameter. This is at least an order of magnitude larger than axons in other classes of echinoderm where they are very small (>1 μ) (Cobb, 1970; Cobb and Stubbs,

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1981; Stubbs and Cobb, 1981; Cobb and Stubbs, 1982). Although these cells are giant by echinoderm standards, they are still small relative to many in other invertebrate groups. These large neurons have allowed consistent extracellular recordings to be made of unitary activity, and thus make possible investigations into the mechanisms of behavior coordination and the sensory abilities of echinoderms (Brehm, 1977; Stubbs, 1982; Moore, in press, Moore and Cobb, 1985).

Each arm in an ophiuroid brittlestar contains a radial nerve cord with a swelling in each segment in the form of a complex ganglion (Fig. 1a). There are three major features to each ganglion: predictably located areas of neuropile, large axons running transversely, and large axons running longitudinally (for illustrations see Cobb and Stubbs, 1981). The neuropile is characteristic of echinoderms (see Cobb, 1970; Cobb and Pentreath, 1978) and consists of small (1 μm or less) varicose axon endings filled with vesicles. There are no specialized synapses reported in echinoderms (Cobb and Pentreath, 1977). Previous anatomical studies of the 'giant' fibers failed to indicate either electrical or chemical synapses between them despite extensive serial section searches (Cobb and Stubbs, 1981). Each ganglionic swelling is joined to that of the next segment by a narrower region of the nerve cord containing only longitudinally orientated axons. The circumoral nerve ring contains giant axons which run circumferentially, connecting the radial nerve cords; it does not show the structure indicative of a complex ganglion (Cobb and Stubbs, 1982).

Associated with each ganglion swelling of the ectoneural radial nerve cord is the hyponeural tissue. This lies aborally and consists of two discrete groups of about 50 giant motor neurons either side of the midline. The hyponeural tissue of a particular segment is not connected to adjacent groups of neurons in hyponeural tissue in segments on either side. The hyponeural system is entirely motor in its function and its presence in the various classes of echinoderms is correlated with major skeletal muscle systems. It is unique in that it's mesodermal; it does not connect directly with the ectoneural nervous system. The two nervous systems are separated by a continuous layer of connective tissue which varies in width between several microns and about 40 nm. This is a true basement in the sense that it occurs between ectoderm and mesoderm cell layers. In regions where the width is above 40 nm there are substantial amounts of collagen fibrils with a basal lamina on either side. Where the basement membrane is thinnest at 40 nm, only the basal lamina is present. Figures 1a and b summarize these anatomical details.

The ectoneural nervous system is unusual in that it does not form neuromuscular junctions directly with most classes of muscle cells. The two main movement-producing systems in echinoderms which are muscle-operated are the tube feet of the water vascular system, and the skeletal system associated with articulating calcite ossicles. Anatomical evidence suggests that the muscles of the tube feet are controlled by a transmitter released across a connective tissue basement membrane from endings of nerves of the ectoneural system (Cobb, 1970; Florey and Cahill, 1977). The skeletal muscles are directly innervated by hyponeural motor nerves. Previous intracellular studies have shown that these skeletal muscles are innervated by the hyponeural motor system at one end, and that they propagate typical action potentials which are triggered by a typical junction potential (Cobb, 1968; Pentreath and Cobb, 1972). Degeneration and general morphological studies on the present species have shown that each giant motor neuron passes to the muscles to be innervated (the intervertebral muscles) and then divides to innervate one end of large numbers of small (5–10 μm diameter) smooth muscle cells (Stubbs and Cobb, 1981). The motor endings are varicose and substantially smaller in diameter than the main axons; they do not form specialized neuromuscular junctions (see Cobb

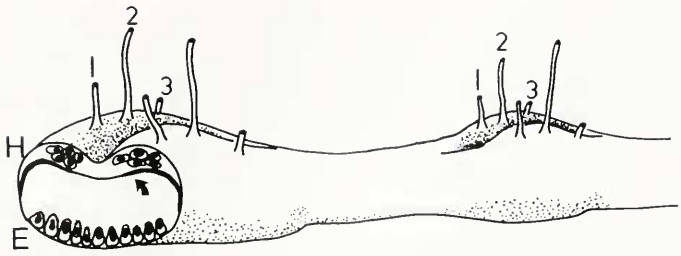

FIGURE 1a. The nervous system of a brittlestar consists, in part, of a circumoral nerve ring and five segmentally ganglionated nerve cords. Two such segmental ganglia are illustrated diagrammatically with one cut in transverse section. The cell bodies of the ectoneural nervous system (E) occur in a layer on the oral surface. The hyponeural motor neurons (H) have cell bodies in aboral swellings either side of the midline. The two nervous systems are separated by a connective tissue basement membrane of varying thickness (arrow). There are three main hyponeural motor axon branches (1, 2, and 3) arising from the swelling on either side of the mid line. These branches ascend aborally to innervate the large intervertebral muscles and to innervate connective tissue (see Wilkie, 1984). The detailed anatomy of this system is illustrated in Stubbs and Cobb (1981).

and Laverack, 1967; Pentreath and Cobb, 1972). Cobb and Pentreath (1976) described the fine structure of the chemical synapse across the basement membrane between ectoneural nerves and the hyponeural motor nerves. They showed that the basement membrane consists, over wide areas, of a thin basal lamina approximately 40 nm thick. The immediately adjacent ectoneural tissue is composed of a continuous layer of axon varicosities filled with vesicles. The hyponeural tissue adjacent is composed of small diameter nerve processes. Cobb and Pentreath proposed that a transmitter was released across the basement membrane by the accepted process of pre-synaptic vesicle exocytosis.

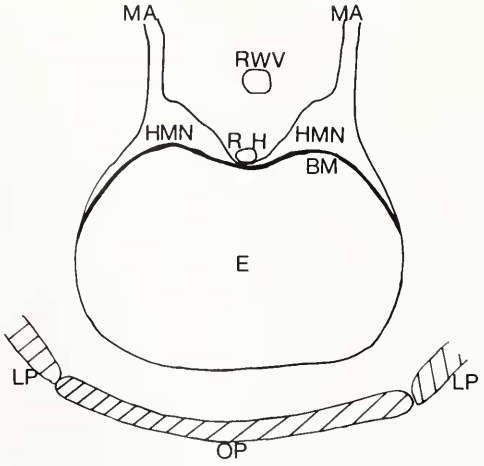

FIGURE 1b. Diagram of a transverse section through the oral part of the arm of a brittlestar. The ectoneural (E) radial nerve cord is covered by the epineural sinus which is enclosed by the oral plate (OP) and part of the lateral plates (LP). A connective tissue basement membrane (BM) separates the hyponeural motor neurons (HMN) from the ectoneural tissue (E). Two motor axon (MA) bundles to the intervertebral muscles are shown as are the radial hemal sinus (RH) and the radial water vascular canal (RWV).

The system of giant fibers in both the ectoneural and hyponeural systems of an ophiuroid has enabled intracellular recordings to be made. This report describes the way motor responses are produced to propagated patterns of activity by interaction between the ectoneural and hyponeural nervous systems.

Materials and Methods

Large specimens of *Ophiura ophiura* were purchased from Millport Marine Station, Isle of Cumbrae. A single arm preparation was used and dissected from the oral surface to expose the ectoneural nerve cord or from the aboral surface for the hyponeural motor nerves.

Extracellular recordings were made using conventional polythene tipped suction electrodes drawn to a tip diameter of 200–500 μ. The two connective tissue sheaths, which in effect form the epineural sinus, were removed but otherwise the nerve cords were left *in situ*. Impalements were made with electrodes prepared from 1 mm diameter thin-walled glass tubing and pulled on a Campden 753 electrode puller. For intracellular dye injection, electrodes were filled with a 5% solution of Lucifer Yellow CH in 3 *M* LiCl and iontophoresed using 500 ms duration, 10 n amp, hyperpolarizing pulses applied at 1 Hz. Most preparations were examined fresh but some were fixed in 5% formol saline for 10 minutes and subsequently dehydrated and cleared in methyl salicylate. Physiological records were obtained using standard apparatus.

Three different methods of stimulation were used to produce propagated activity within the ectoneural tissue of the radial nerve cords:

(1) Photic stimulation was achieved by extinguishing a spot of light directed at a few peripheral segments on the arm. This action typically produces a burst of spikes which is conducted through the ectoneural nervous system (Stubbs, 1982; Moore and Cobb, 1985).

(2) Chemical stimulation was produced using solutions of the amino acids L-leucine or L-lysine at concentrations of 10^{-10} applied with a dropper to the tip of the arm. This produced conducted activity within the radial nerve cords (see Moore, in press).

(3) Electrical stimulation: a suction electrode containing two insulated silver chloride wires was applied to an exposed peripheral part of the nerve cord. This stimulation produced unpredictable but large bursts of activity conducted within the ectoneural system. This stimulation, although non-specific, clearly was useful in that it produced substantial numbers of synaptic potentials within the hyponeural motor neurons.

Interpretation of intracellular recordings

Intracellular recordings from echinoderm neurons have not been reported previously. It is therefore necessary to establish the criteria for judging success with intracellular recordings and iontophoretic dye fills.

The main criterion for a successful cell impalement is a stable and appreciable negative resting potential. With these cells, resting potentials of at least −25 mV, and on occasion as much as −60 mV were recorded. All data reported here were obtained from 40 mV or better impalements. Lower resting potentials are usually associated with injury discharge, which is characterized by a continuous, high-frequency train of small, positive-going potentials. Larger resting potentials indicating better impalements show spikes up to 70 mV in amplitude, which, with a resting

potential of −60 mV, implies a 10 mV over-shoot of zero. Such cells can be caused to spike using injected depolarizing current and, on rebound from hyperpolarizing current, they will also sometimes show spikes on stimulation of the peripheral radial nerve cord. These spikes are always of constant amplitude and timecourse. Spontaneous fluctuations in membrane potential of varying amplitude with a slower risetime than spikes were interpreted as synaptic potentials.

Lucifer Yellow was injected by iontophoresis into many neurons in which penetrations were deemed successful by the criteria outlined above. The period of dye injection varied considerably from cell to cell, and in some cases the cells were apparently only partially filled with dye. It is difficult to be certain that a fill is complete but fills were deemed partial if the dye faded in intensity to the point of invisibility while the neuron process remained relatively large in diameter. In contrast, fills were deemed complete if the Lucifer remained at high intensity while the neuron process itself diminished in diameter to the point of invisibility.

Results

Morphology of the ectoneural neurons

Forty ectoneural cells were partially or completely filled with dye. All but three of these fills were of neurons that run longitudinally and complete fills showed that some of these neurons passed through two complete segments with a terminal plexus of varicosities at each end. The cell body is found at one end of the axon close to the varicose plexus (Fig. 2b.). Many of the fills, however, were partial with the cell body and varicosities visible at one end but the axon fading before reaching the other terminal region. In some fills the cell body was on the opposite side of the midline to the longitudinal axon. The remaining three cells were completely filled and showed a transverse orientation, with the main axon running across the ganglion and showing terminal varicosities either side of the midline (Fig. 2a.). The region of terminal varicosities in both types of neuron covers a relatively large area of each ganglion. This finding explains the failure to find synapses of any sort between the giant fibers themselves in the previous anatomical study and may well be of significance in understanding how integration takes place in the nervous system. The finding of longitudinal and transverse axons also fits the previous anatomical data. The varicose endings in some preparations were in a two dimensional layer at the depth of the basement membrane between the two nervous systems and this again fits the previous anatomical evidence of a complete layer of small varicose vesicle filled profiles in this region.

Physiology of the ectoneural neurons

The ectoneural cells are loosely packed and are difficult to impale successfully for long periods. Impalements were made in both nerve cell bodies and axons (as the cell bodies are invariably on the surface, and the axons deeper, they are distinguishable). A −60 mV impalement produces a spike of approximately 70 mV amplitude, *i.e.*, it overshoots zero by about 10 mV (Fig. 3c.). Some penetrations (about 1 in 50) are silent with a resting potential of −25 mV or greater. These cells show no signs of synaptic potentials or injury potentials but will spike when depolarizing current is injected and also on rebound from hyperpolarizing current. Some will also spike when the nerve cord is stimulated peripherally (Fig. 3b.) and this is concomitant with extracellularly recorded spikes using a suction electrode. A

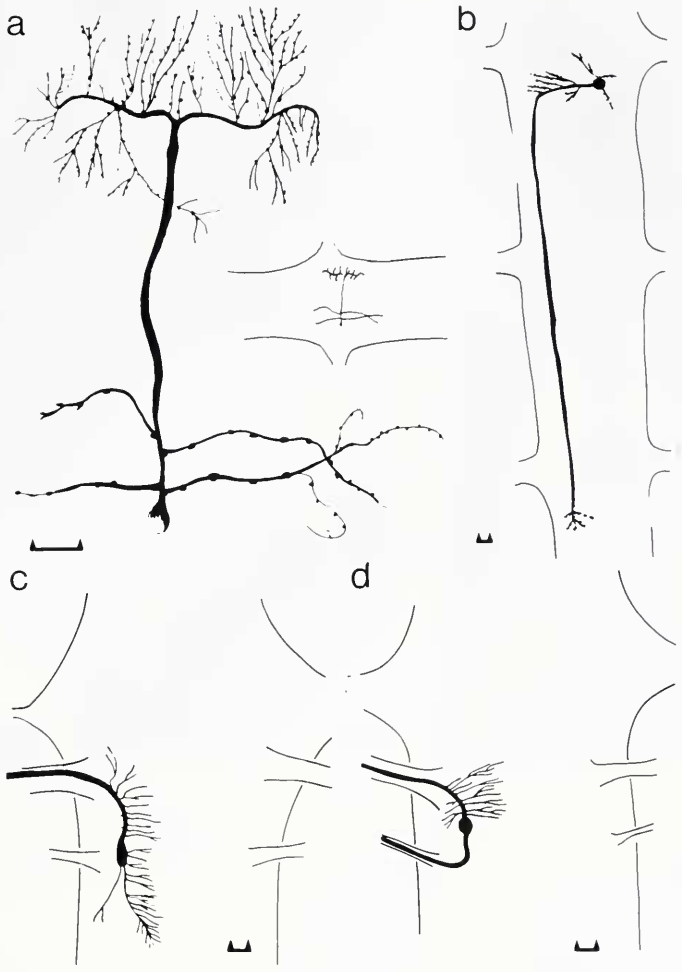

FIGURE 2a. Camera lucida drawing of a neuron filled with Lucifer Yellow in the ectoneural system, lying transversely across the radial nerve cord. Note the varicose terminals of branches. (Inset) The relationship of the neurone to a segment of the nerve cord. Scale = 20 μ. b. Ectoneural neuron showing varicose terminals lying longitudinally within three segments of the radial nerve cord. Scale = 20 μ. c. Hyponeural neuron showing non varicose fine branches that are post-synaptic dendrites. A single axon lies in the largest motor nerve branch to the muscles of the vertebral ossicles. The outline of a single segment is shown. Scale = 20 μ. d. Hyponeural neuron similar to 2c, but with axons in both motor branches to the vertebral ossicles. The outline of a single segment is shown. Scale = 20 μ.

small number of cells showed synaptic potentials and did not spike either to injected current or spontaneously (Fig. 3a.).

It is not possible with the small number of fills achieved at present to correlate structure with function, beyond the observation that the longitudinal axons show spike potentials and few synaptic potentials.

Anatomy of the hyponeural neurons

Usually, the fine dendritic processes arise directly from the cell body with the minimum of branching but generally cover quite a large area of the ganglion. One

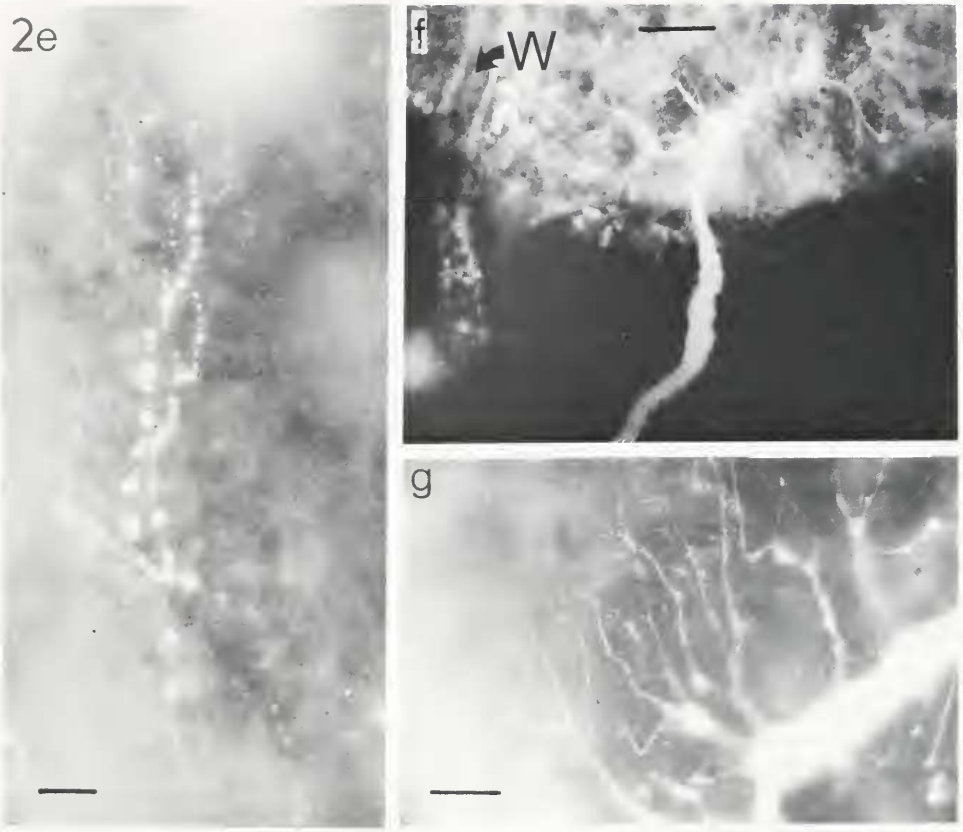

FIGURE 2e. Varicose endings in part of the Lucifer Yellow filled ectoneural neuron illustrated in 2a. Scale = 10 μ. f. Dye-filled hyponeural neuron showing axon dissected free from ossicles. The non-varicose endings cover about 1/3 of the ganglionic swelling on one side of the midline. The segmental branch of the water vascular system is shown (W arrow). This cell is similar to that illustrated in 2c. Scale = 40 μ. g. Detail of non varicose processes from a dye-filled hyponeural neuron. Note the fine branches leave the main body of the neuron directly. Scale = 20 μ.

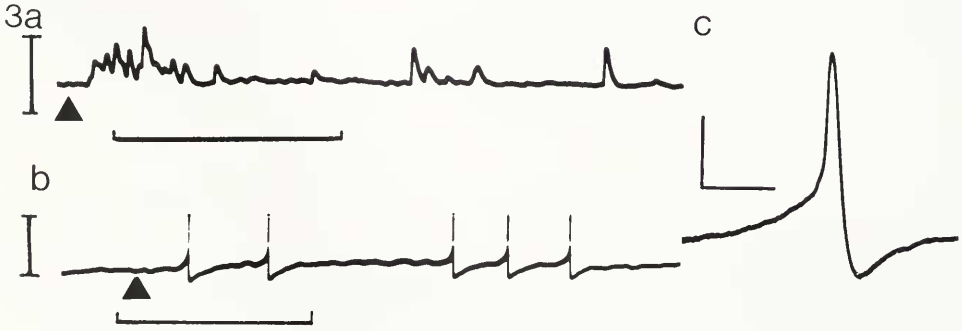

FIGURE 3a. Intracellular impalement of an ectoneural neuron showing post synaptic potentials to a photic stimulus to the arm tip (arrow). This cell did not show spike potentials when depolarising current was injected. Very few impalements of this type of non spiking cell in the ectoneural nervous system have so far been made. b. Longitudinal neuron penetration showing typical spike potentials to a photic stimulus (arrow) to the arm tip. Time scale = 1 s, vertical scale: a = 10 mV, b = 20 mV. c. Intracellular spike potential to photic stimulation filmed at a higher sweep speed to illustrate basic shape. Time scale = 20 ms, vertical scale = 20 mV.

hundred cells were filled with Lucifer Yellow and in no preparation were even the finest processes found to cross the midline or pass to the two adjacent segmental ganglia along the nerve cord. The cells appear either monopolar or bipolar in the sense that they give off either one or two large axons. These axons pass up one or two of the three motor trunks (see Fig. 1a). It is not possible to dissect the entire axon of a motor neuron away from the ossicle to show the region of the neuromuscular junctions, and any sectioning technique requires lengthy decalcification. However, the details of the neuromuscular junction have been worked out using other techniques (Stubbs and Cobb, 1981). The substantial dendritic branching of the hyponeural neurons immediately adjacent to the cell bodies are not typical of invertebrate motor neurons and more closely resemble some type of neuron from vertebrate C.N.S.

Lucifer Yellow was successfully injected into more than 100 hyponeural motor cells. This was deemed sufficient, because of the consistency of results, to identify the general morphology of these cells.

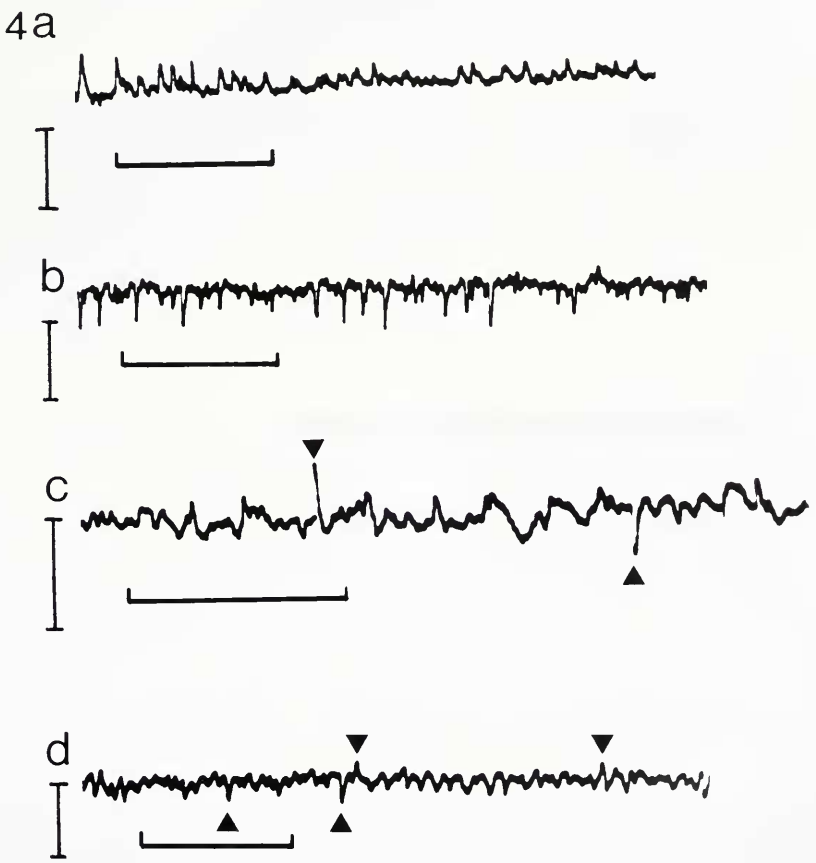

FIGURE 4a–d. Intracellular recordings from single hyponeural neurons showing the characteristic synaptic potentials present in all of many hundreds of impalements. The size and frequency of such events varied with time and from preparation to preparation. All cells impaled in a particular preparation at a particular time tended to show mainly either excitatory (4a) or inhibitory (4b) potentials. Long-term observation of such impalements occasionally show the opposite inhibitory or excitatory synaptic potentials (4c, d arrows). These events are caused by variable pre-synaptic activity in an unidentified class of ectoneural interneuron. Time scale = 1 s, vertical scale = 10 mV.

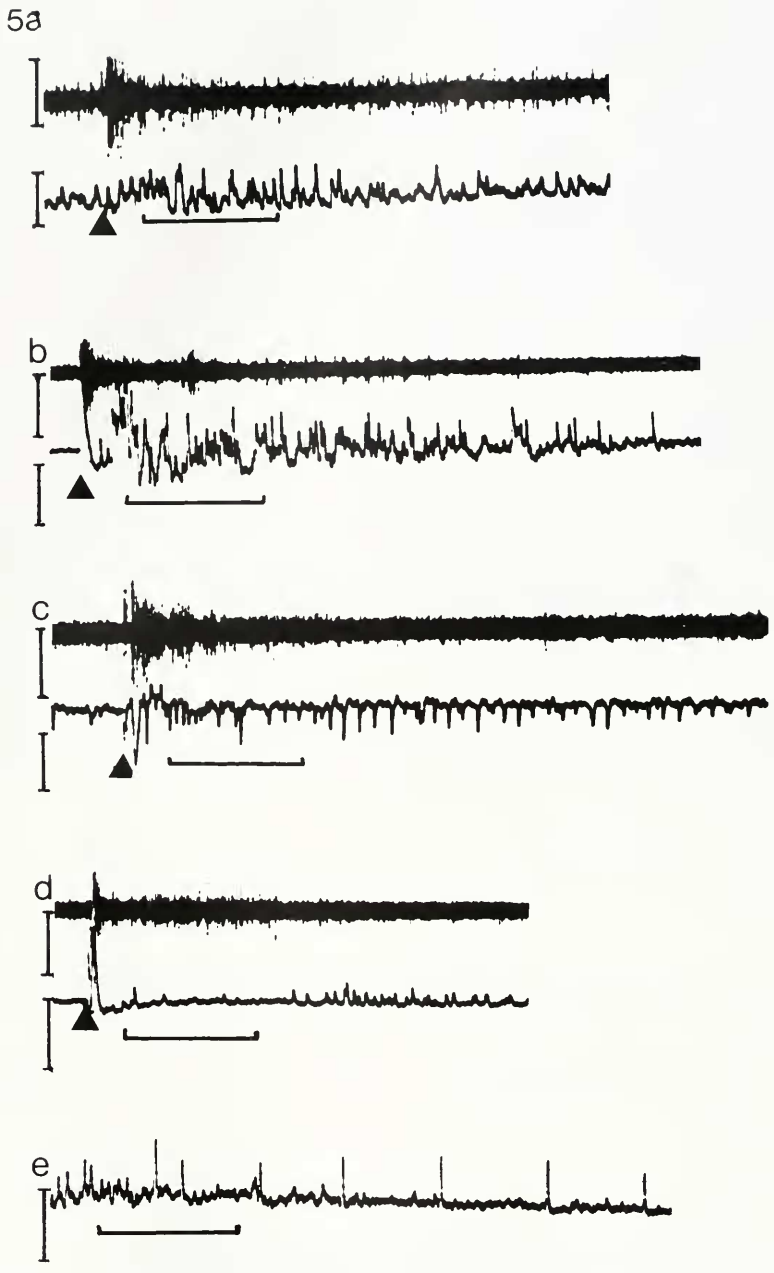

FIGURE 5a–e. Upper trace, extracellular record from ectoneural radial nerve cord showing the propagated burst of spike activity to photic or electrical stimulation (arrow) of the nerve cord six segments distal but which shows a similar pattern where ever it is recorded; lower trace, intracellular record of post synaptic potentials within a hyponeural nerve cell body. a. Excitatory post synaptic potentials to photic stimulation, note summation of potentials cause slight depolarization. b. E.p.s.ps to electrical stimulation, note more substantial summation causing depolarization. (There is an initial stimulus artifact when electrical stimulation is used.) c. Mainly i.p.s.ps.; note some initial summed hyperpolarization, then small e.p.s.ps. and finally a much increased rate of i.p.s.ps., (electrical stimulation). d. Summed e.p.s.ps. followed

Physiology of the hyponeural neurons

The hyponeural cells are easier to impale compared to ectoneural neurons. The point where the motor axons leave the ganglion can be impaled with careful dissection. When the region of the cell bodies is impaled injury potentials are never observed but the resting potential (−30 to −60 mV) always shows some small continuous fluctuation. These are present even in behaviorally quiescient preparations but in many cases are clearly inhibitory and excitatory synaptic potentials (Figs. 4a, b.). These cell bodies do not spike to injected depolarizing current or show rebound spikes to hyperpolarizing current. These hyponeural cell bodies do show a burst of synaptic potentials coincident with the burst of spike activity in the ectoneural tissue in response to stimulus. The synaptic potentials may be excitatory or inhibitory and can summate to give potentials up to 15 mVs (Figs. 5a–c.). Impalement of the motor axons often causes injury spike potentials and these axons can also be induced to spike by stimulation of the ectoneural system.

There is a tendency for a particular cell at a particular time to show either excitatory or inhibitory potentials but sometimes they show both (Figs. 4a–d.). Many hundreds of impalements on different preparations, which could be kept alive for 10–12 hours and stimulated using the three different parameters, show that the presence of excitatory or inhibitory synaptic potentials varies with preparation, time, and stimulus and is unequivocally a consequence of presynaptic ectoneural activity. Thus there is only one class of hyponeural motor neuron. The cell body region which is normally impaled is non-excitable and spikes are initiated in an axon hillock region. The motor axons can also be impaled (Fig. 6a.). Some penetrations of cell bodies showed large rapid depolarizations which may be tonically conducted axon spikes which penetrate the cell soma but there is no absolute criteria to distinguish them from synaptic potentials (Fig. 5e.).

Each hyponeural motor neuron therefore receives both excitatory and inhibitory synaptic input across the basement membrane from ectoneural neurons. This synaptic input can, however, never be correlated directly with units in the propagated spike potentials that can be recorded extracellularly in the ectoneural systems (Fig. 5d.). The extracellular electrodes cover most of the nerve cord and it is likely that unitary potentials would be recorded from most large, longitudinally conducting, ectoneural axons. This lack of correlation implies that another class of interneuron within the ectoneural system is interposed between the longitudinally conducting neurons and the hyponeural motor neurons. The extracellular electrode is placed one segment peripheral to the ganglion impaled but the pattern of spikes recorded is similar in each segment (see Stubbs, 1982). The failure to record activity from this other class of ectoneural interneuron may be due to a smaller size of axon or even that these neurons do not spike.

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by a lowered level of activity, then increased e.p.s.ps. The ectoneural activity recorded extracellularly is in general coincident with synaptic activity but there is no correlation with individual spikes. This is an extreme example since the main burst of synaptic activity is coincident with ectoneural activity only marginally above the non-stimulated state. This implies at least one other class of ectoneural interneuron is interposed between them. Time scale = 1 s, vertical scale = 40 μV (extracellular), 10 mV (intracellular). e. Activity from a stimulated preparation showing synaptic activity. The single large units may be attenuated soma spikes propagated tonically from the spike initiating site. Such potentials are common, especially to electrical stimulation, but there is no absolute criteria to distinguish them from large e.p.s.ps. Time scale = 1 s, vertical scale = 10 mV.

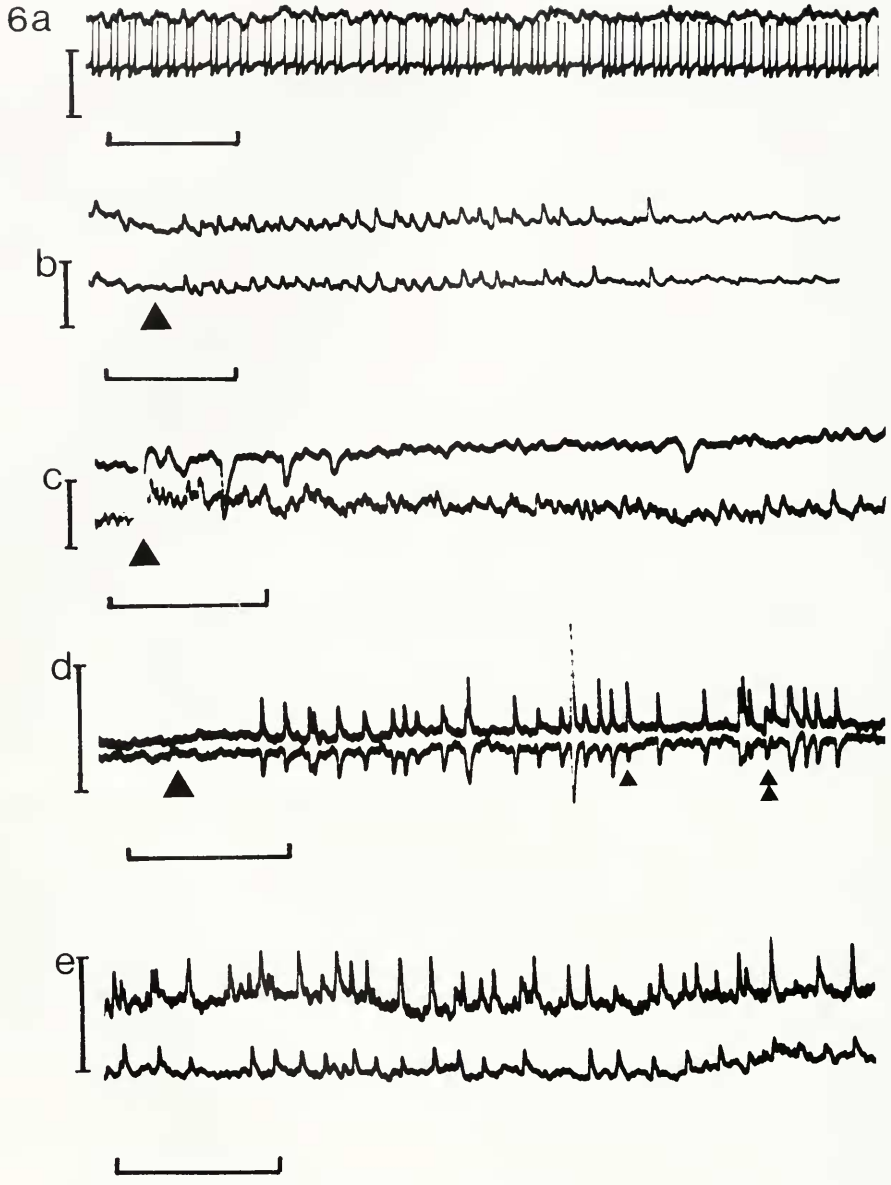

FIGURE 6a. Responses recorded intracellularly in two separate hyponeural motor neurons. Upper trace, small irregular post synaptic potentials from cell body; (bar = 5 mV); lower trace, spontaneous spike potentials from close to axon hillock region (bar = 40 mV). Spikes were never recorded from the cell body region either spontaneously or to injected current. b. Two cells impaled close together in the same segmental ganglion ipsilaterally (photic stimulation arrow). Injected current showed that these were not recorded from a single cell and repeated impalements of other cells in same area of such hyponeural preparations showed a strong correlation between recorded synaptic activity implying many of these cells received input from the same presynaptic units. c. Similar to (b) but cells impaled at opposite ends of a single ganglion ipsilaterally. In general the closer cells are together the greater the correlation in pattern of p.s.ps. (electrical stimulation arrow). d. Two cells impaled contralaterally in the same ganglion both with 60 mV resting potentials. One cell shows e.p.s.ps. and the other i.p.s.ps. Although the correlation is striking it is not an exact mirror image; analysis of responses over a number of seconds shows occasional

Experiments were initiated using two intracellular recording electrodes to compare responses of hyponeural motor neurons in different anatomical positions. Two cells were impaled simultaneously at varying distances apart within the same ganglion including the electrodes being on opposite sides of the midline. Cells in adjacent segmental ganglia were also impaled simultaneously, and in all cases responses to the same stimulus were recorded. Initially all cells impaled in dual electrode experiments were tested with 10 n amps of both depolarizing and hyperpolarizing direct current. In none of these experiments was there any sign that the same cell was impaled twice nor was there any indication that cells were electrically or chemically coupled. In these experiments, the cells of the pair where current was not injected showed no short or long-term changes in resting potential or change in the rate, polarity, or size of synaptic potentials already present. Previous anatomical studies of this region (Cobb, 1970; Cobb and Pentreath, 1976; Stubbs and Cobb, 1981) have not provided any evidence for such synapses at the ultrastructural level.

Experiments with two microelectrodes show that the closer the neurons are together, ipsilaterally in one segment, the greater the correlation in the pattern of junction potentials to stimulus (Figs. 6b, c.). If cells on opposite sides of the midline in the same ganglion are impaled simultaneously, then one cell may show purely e.p.s.ps. and the other i.p.s.ps. (Fig. 6d.). Cells impaled in sequential ganglia ipsilaterally show a looser correlation in the pattern of junction potentials (Fig. 6e.).

Discussion

This study is the first stage in developing an understanding of function in the echinoderm nervous system at the cellular level. It has demonstrated the basic morphology of some echinoderm neurons. It has shown that there are fine plexuses of varicose endings at the terminal region of the large ectoneural interneurons. Lucifer fills have also confirmed the electron microscopical evidence for longitudinally and transversely orientated neurons. More work is required to show if these are the only major classes of neurons and to what extent they are divided structurally and functionally into further subclasses.

The manner in which echinoderm neurons make contact synaptically has not been well documented. The unusual contact between ectoneural and hyponeural neurons across the basement membrane shows no membrane specialization associated with it (Cobb and Pentreath, 1976). There are also no membrane specializations at any other chemical synapse between neurons or at neuromuscular junctions (see Pentreath and Cobb, 1972). There have been no published reports of electrical synapses that show the characteristics of gap junctions. Previous anatomical work on the giant fiber system (Cobb and Stubbs, 1981; Stubbs and Cobb, 1981) showed, using serial section analysis and electron microscope evaluation of critical sections, that the individual giant fibers could not be traced for long distances. They failed to show any structure which might be interpreted as either a chemical or an electrical synapse onto another neuron. By comparison with other invertebrate giant

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differences in amplitude (single arrow) and pattern of events (double arrow), electrical stimulation arrow. There is no evidence of any hyponeural axon crossing over between contralateral sides of the ganglia from Lucifer fills; these are undoubtedly separate cells. e. Two cells impaled in sequential segmental ganglia showing some correlation in patterns of synaptic activity as a burst of propagated activity caused by a remote previous chemical stimulus is conducted from ganglion to ganglion through the ectoneural nervous system. Time scale = 1 s, Vertical scale = 10 mV.

fiber systems, large electrical synapses might have been anticipated. The present study has, however, shown that the terminal regions at both ends of ectoneural and hyponeural neurons consist of a fine plexus of neuronal processes. In the case of the hyponeural motor neurons the present evidence shows that these fine processes represent a functional connection with ectoneural neurons.

It seems likely that many impalements with low resting potentials are poor quality with current leakage as confirmed by predictably smaller but undoubted spike potentials. Takahashi (1964) reported extracellular unitary potentials using metal-filled glass microelectrodes in the small fibered nervous system of an echinoid. This work has never been repeated despite very substantial efforts by the author and others using a wide range of refined extracellular electrodes. Suction electrodes, only, have been used successfully by Brehm (1977) and in this laboratory (Stubbs, 1982; Moore, in press; Moore and Cobb, 1985) to record unitary potentials extracellularly, and then only from the giant fibers of ophiuroids. It is therefore very unlikely from this evidence, and from the characteristics of impalements described in an earlier section, that extracellular field potentials from distant spike potentials could account for any of the synaptic activity described in the present paper. Even using small suction electrodes, the largest spikes ever recorded were a few tens of microvolts, and synaptic potentials recorded intracellularly from cells with a resting potential of 30 mV or more were several millivolts.

Function in the echinoderm nervous system can be simplistically divided into three parts. First is the neural mechanism that produces the pattern of behavior coordinating activity due to the local sensory input into a single ganglion. Next is the neuronal function that transmits coordinating activity from ganglion to ganglion. The final step is the production of motor responses within the hyponeural system at each segment in response to the through conducted patterns of activity. This report provides a preliminary description of the anatomy and physiology of this last part of these three general levels of integration. Figure 7 is a summary diagram of the structure of the neurons involved.

The hyponeural motor neurons are either monopolar or bipolar with fine non-varicose dendritic branching and the cell bodies are non-excitable. The connection between the two nervous systems has been unequivocally demonstrated as a pre-synaptic plexus of small ectoneural endings adjacent across the basement membrane to a post-synaptic plexus of small hyponeural dendrites. This fits the previously somewhat puzzling findings of electron microscopical studies. Physiological connection between the two nervous systems has been demonstrated for the first time and shows that coordinated inhibition and excitation of the hyponeural motor nerves could, for example, produce the rapid arm flexures associated with escape behavior. This situation of a chemical synapse across a basement membrane is similar to that proposed to account for the innervation of the muscles of the ampullae and tube feet. There is anatomical evidence for this (Cobb, 1970; Florey and Cahill, 1977), and recently Florey and Cahill (1980) carried out a mathematical analysis of the various factors involved and showed that although the connective tissue in the tube foot can be as thin as 4–5 μ, transmission is feasible across as much as 25 μ onto the muscle cells. In the present situation only a 40 nm basal lamina is present over much of the ectoneural/hyponeural boundary, but in some regions the basement membrane can be several microns thick and is composed of collagenous connective tissue. The reason why a separate mesodermal nervous system has evolved and why the ectodermal nervous system never penetrates any mesodermal tissue is enigmatic. This poses a fascinating developmental question as to how the pre- and post-synaptic elements achieve the correct positional geometry separated by an uninter-

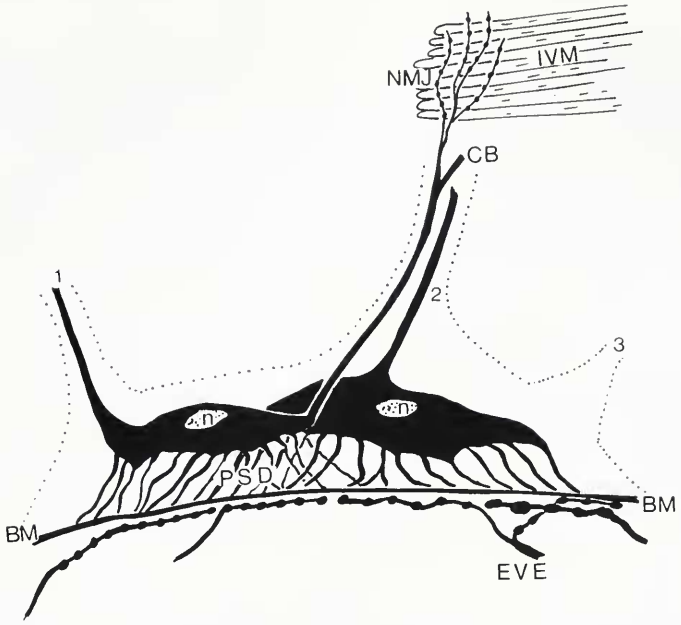

FIGURE 7. Summary diagram of structure of hyponeural motor neurons. Small varicose endings (EVE), form unspecialized synapses against a continuous connective tissue basement membrane (BM). Hyponeural motor cell bodies (n = nucleus) have a large plexus of post synaptic dendritic (PSD) processes which abut against the basement membrane. The motor cells are either unipolar or bipolar sending one or two branches up the three (1, 2, and 3) axon trunks to the intervertebral muscles (IVM). The motor axons branch, and also innervate the connective (CB) tissue via the juxtaligamentous ganglion. It is not known whether there is a separate population of motorneurones involved in this innervation or whether, as illustrated, the same neurons branch and reduce in size to form single varicose ending type of neuromuscular junction (NMJ). Not to scale.

rupted basement membrane. It has also been shown that whatever the reason for two totally separate nervous systems, the hyponeural is excited or inhibited in a relatively straight-forward way by the ectoneural system. That there is no direct correlation between individual units in bursts of ectoneural spikes that are conducted on through the nervous system and synaptic potentials in the hyponeural neurons implies that there is, at least, a second class of interneurons interposed between these two systems. These unknown interneurons can both excite or inhibit all the hyponeural motor axons in each ganglion depending on levels of excitation present. The records using two electrodes in the hyponeural cells in different positions show these ectoneural interneurons to have a fascinating and perhaps unique mechanism of function and overlapping fields of synaptic activity. It is not, however, worthwhile to speculate as to how coordinated motor output is achieved until more is known about these ectoneural interneurons. It is clear that the key to understanding the unique non-centralized nervous system of echinoderms lies in defining interneuron function within the ectoneural system.

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